An Experimental investigation of the lethality of hydrogen sulphide

AN EXPERIMENTAL INVESTIGATION OF THE LETHALITY OF HYDROGEN SULPHIDE

Alberta
ENVIRONMENT

H_2S Toxicity Analysis

(LA-79-9007)

FINAL REPORT

Submitted August , 1979

by

A. S. Clanachan, Ph.D.

Department of Pharmacology
University of Alberta
Edmonton, Alberta T6G 2H7

CONTENTS

SUMMARY

1. Groups of 10 mice (BALB/CCR strain) were exposed to various hydrogen sulphide concentrations (up to 1300 ppm) for different time intervals (1 to 30 min). Exposure times and concentrations were randomized and the experiments were performed in duplicate. Animals were exposed only once in order to avoid possible errors due to sensitization or tolerance to the gas.

2. Loss of righting reflex EC_{50} (the concentration of gas which will produce unconsciousness in 50% of the test subjects) and LC_{50} (the concentration of gas which will kill 50% of the test subjects) were calculated for each exposure duration. All survivors were retained for at least a further 24 hours and any additional deaths were noted.

3. The results indicate that the EC_{50} for loss of righting reflex (Loss RR) and the LC_{50} are indeed time-dependent. The concentration of hydrogen sulphide required to produce unconsciousness or death was greater at the shorter exposure durations (< 15 min). However, the Loss RR-EC_{50} and LC_{50} appeared to become less dependent on time at the longer exposure durations (≥ 15 min).

4. No additional deaths were noted in any group of survivors observed for periods up to 5 days following exposure.

INTRODUCTION

Quantitative studies concerning the acute toxicity of hydrogen sulphide (H_2S) appear to have received little attention. The literature describing the biological effects of H_2S contains many reports describing isolated clinical findings in patients who accidentally became exposed to toxic levels of the gas. However, although the clinical signs and symptoms of H_2S poisoning are fairly well documented, the concentrations of the gas to which the patients were exposed could not be accurately determined. Also, in many cases, the duration of exposure was not recorded. Consequently, the potential interaction between time of exposure and concentration of exposure has not been fully explored.

There are some reports in the literature concerning this potential interaction. The body has a large capacity for detoxifying sulphide (Haggard, 1925; Weber and Lendle, 1965) and this has led to the belief that the toxicity of H_2S is more closely related to concentration than to duration of exposure. O'Donoghue (1961) supported this idea. In an investigation of the toxicity of H_2S in swine and rabbits, the method used was designed to place the animals in the gassing chamber and increase the H_2S concentration over a period of time. However, on the basis of a sudden accidental exposure of one pig and three rabbits, he concluded that the concentration

was the more important factor. The pig was exposed to 400ppm for 1 second - (which proved to be a fatal exposure); the three rabbits were suddenly exposed to 1000ppm (one died and the other two had no after effects). Although this study indicates that the rate of rise of the H_2S concentration may influence toxicity, it does not critically examine the relationship between the time and concentration of exposure on the lethality of the gas.

This experimental study was performed in order to investigate the potential interaction of the exposure duration and exposure concentration on the acute toxicity of H_2S. Of particular interest was the examination of this interaction at short (1-15 min) exposure durations as this was in the range of the potential exposure time following a pipeline fracture (Whittaker and Wilson, personal communication).

METHODS

(a) Gassing Chamber

A gassing chamber was designed and constructed to permit sudden exposure of the mice to each H_2S concentration. Also, rapid removal of the mice from the gas was considered necessary in order that accurate data could be obtained at the low exposure durations. The chamber (Fig. 1), constructed of clear plexiglass, consisted of two chambers. The larger one (volume 120 l) served as the chamber containing the H_2S gas, whilst the smaller chamber (volume 5 l), which could be sealed off from the larger chamber, served as an entry and exit mechanism for the mice. A smaller wire cage, constructed on slides, could be moved between the smaller chamber and the larger chamber. Groups of mice were initially placed in the wire cage in the smaller chamber which contained air. The wire cage was then positioned, for various time periods, in the larger chamber containing H_2S. In order to remove the animals from the gas, the wire cage was quickly repositioned in the smaller chamber. The smaller chamber was then automatically sealed off from the H_2S and flushed with normal air (50 l min^{-1}). This procedure effected a rapid (10 sec) removal of the mice from the H_2S.

(b) Preparation of H_2S concentrations (ppm range)

Various H_2S concentrations were prepared by dynamic dilution, i.e. by mixing two accurately known flows of pure gas (air and H_2S).

Air, obtained from the building compressed air supply, (line pressure = 25 psi) was passed through calibrated flow meters and also a dry gas meter (previously checked against a wet gas meter). A flow rate of 20 $l.min^{-1}$ was used throughout the experiments. Pure (99.5%) H_2S gas was obtained from Matheson, Canada Ltd. The flow rate was adjusted by a micro NRS metering valve (line pressure= 35 psi) and monitored by a flow meter and a soap bubble flow meter.

This apparatus is considered as a very accurate method of preparing known mixed gas concentrations and is sometimes used to prepare calibration gases for more sophisticated instrumentation, eg. gas chromatographs. Various H_2S gas concentrations were prepared as follows:

Final Concentrations (ppm v/v)	H_2S ($ml.min^{-1}$)	Air ($l.min^{-1}$)
600	12	20
700	14	20
800	16	20
900	18	20
1000	20	20
1100	22	20
1200	24	20
1300	26	20

The two gas flows were allowed to mix thoroughly before passing into the gassing chamber. The gassing chamber was then allowed to equilibrate with each H_2S concentration for at least 45 min. before the exposure of each group of animals.

(c) Experimental subjects

Equal numbers of male and female mice (BALB/CCR strain) were used in this study. The animals were approximately 5-6 weeks of age (15-25g). Animals were used in groups of ten and each group was exposed to only one time-concentration combination in order to avoid errors due to potential tolerance or sensitization to the H_2S. Exposure times and concentrations were randomised. Experiments were performed in duplicate to give a total of 20 animals per time-concentration combination.

(d) Measurement of toxicity

Following exposure, two indices of acute toxicity were observed.

(i) Loss of Righting Reflex (Loss RR)

An animal is said to have lost its righting reflex if it fails to right itself after having been placed on its back. Loss of righting reflex serves as a measure of unconsciousness. Loss of righting reflex was estimated immediately following the return of the animals to air. Loss RR-EC_{50} (the concentration of

H_2S required to make 50% of the test subjects unconscious) was calculated for each exposure duration. Similarly, Loss RR-ET_{50} (the time of H_2S exposure that is required to make 50% of the animals unconscious) was calculated for each exposure concentration.

(ii) Death

The number of animals that died during exposure was measured. LC_{50} (the concentration H_2S that kills 50% of test subjects) was calculated for each exposure duration. Similarly, the LT_{50} (the time of H_2S exposure that is required to kill 50% of test subjects was calculated for each concentration.

(e) Calculation of results

Loss RR-EC_{50}, LC_{50}, Loss RR-ET_{50}, and LT_{50} were calculated by computer assisted probit analysis. Probit analysis is a well accepted method for toxicity estimation (Bliss, 1935; Finney, 1947; Cook, 1972), and provides not only the 50% value but also an error estimate (Standard Deviation) of this value.

RESULTS

The number of mice in each group that had lost their righting reflex and the number that died during each exposure time-concentration combination are contained in Tables 1 and 2 respectively. Note that animals that were dead following exposure are also included with the numbers that had lost their righting reflex. This is valid because before an animal died at any one time-concentration combination, it first of all was rendered unconscious.

Figure 1 shows the relationship between the percentage of animals killed and the exposure concentration for different time intervals. Figure 2 shows the relationship between the percentage of animals killed and the exposure duration for different exposure concentrations. The death rate was dependent upon both exposure duration and exposure concentration.

Loss RR-EC_{50} and the LC_{50} are presented in Table 3. Loss RR-ET_{50} and LT_{50} are shown in Table 4.

The relationship between LC_{50} and exposure duration is displayed graphically in Figure 4. Both the Loss RR-EC_{50} and the LC_{50} are time-dependent. In both cases, higher concentrations were required at the shorter exposure durations to produce a standard effect (the 50% response). For example, the LC_{50} (± SD) for an exposure duration of 2.5 min was 1734 (± 110) ppm whereas following 30 min exposure the LC_{50} was 961 (± 19). The LC_{50}

showed a greater dependence upon exposure duration at the lower (2.5 - 10 min) than at the higher (> 10 min) exposure durations (Figure 4). Similarly, LT_{50} values were dependant upon the exposure concentration (Table 4, Figure 5).

Animals that survived each exposure were retained for a further 5 days. There were no additional deaths.

DISCUSSION

(a) Methods

The method used to expose mice to various hydrogen sulphide concentrations (500-1300 ppm) for different time intervals (1-30 min) appeared to be satisfactory. The gassing chamber with the small entry/exit chamber permitted the animals to be rapidly exposed to the gas and also to be rapidly returned to normal atmospheric air. The entry/exit chamber also afforded the operator a greater degree of protection from the gas! Thus, the experimental animals received a "square wave" pulse of H_2S. This rapid transfer of the animals in and out of each H_2S concentration greatly increased the accuracy of the exposure durations, expecially at the shorter exposure periods.

Although no accurate or continuous measurement of the hydrogen sulphide concentrations was made, the method used (dynamic dilution) was considered to be sufficiently reliable. Flow rates were monitored continuously with flow meters and checked intermittantly (every 3-4 min) before and during each experimental run with the dry gas meter (air) and the soap bubble flow meter (H_2S). High line pressure and fine control valves, which were used throughout the system, resulted in steady accurate flows, and hence reproducible and accurate concentrations. As a further precaution to avoid systematic errors in

the system, exposure times and concentrations were randomised. Groups of 10 mice were exposed at one time, and as each run was examined in duplicate, a total of 20 animals were used at each time-concentration combination. This number (20) of experimental subjects yielded statistically meaningful results.

(b) Results

The results obtained in this study indicate that the acute toxicity of H_2S is time-dependent. The concentrations of gas required to produce both unconscious and death were greater at the shorter exposure durations. A greater dependence on time was observed at lower (< 15 min) exposure durations.

The rapid spontaneous recovery of surviving mice would seem to indicate that this experimental animal is not well suited to a study of the efficacy of various therapeutic measures following the exposure. It is thus surprising that most of the information supporting the use of sodium nitrite in H_2S poisoning has been obtained using the mouse as an experimental animal (Smith and Gosselin, 1964; 1966). Furthermore, if the animals recovered from exposure, there were no further deaths. This indicates that following a single exposure the mice were not subject to potential fatal complications which required treatment. Although, there were no further deaths, the animals did appear to be "stressed" for 1-2 days following exposure, eg. they showed marked

piloerection. No quantitative assessment of this effect was made.

LC_{50} values, although time-dependent for the time period examined, were confined to a narrow concentration range (961-1734 ppm). Also, the mouse population used was homogeneous, and so each displayed an almost identical sensitivity to the gas. This resulted in a sudden onset of toxicity at about 800 ppm (see Fig. 2) and by 1800 ppm all animals were dead. In the human population, where many factors can influence sensitivity (age, drugs, genetic factors, disease, etc.) lethality may extend over much greater concentration ranges. However, the LC_{50} values obtained in the study using mice are within the range reported for man and other species (Sayers, Smith, Fieldner et al., 1925).

(c) Possible future experiments on acute toxicity of H_2S

(i) Completion of present study

It would seem worthwhile to extend the range of time and concentrations examined in order to complete the family of curves in figures 2 and 3. Higher exposure concentrations should be used to complete the curves for 10, 7.5, 5 and 2.5 min exposures. From the present experiments, it appears that only the 2.5 line has a significantly different slope. However, the 2.5 min data are not reliable due to the narrow range of effects observed. Similarly, longer exposure

periods should be done to complete the curves for the lower exposure concentrations. From the work of O'Donoghue (1961) it seems as though a gradual increase in H_2S concentration may afford a degree of protection relative to a sudden exposure. If so, such an effect may have a significance with respect to predicting toxicity following exposure to H_2S gas. Presumably under some circumstances, an individual exposed to H_2S gas would experience a gradually increasing and then decreasing concentration.

(ii) Time-course of toxicity

In this investigation, two very definite measures of toxicity were examined (unconsciousness and death). Similar, but non-quantitative, data has been obtained in many other studies. From a therapeutic standpoint, the study of the time-course of the onset of toxicity would be beneficial. Questions that should be answered include, for example, what is the rate of onset of toxicity? When is toxicity reversible or irreversible? What is the effect of various therapeutic regimens on these parameters? Can H_2S poisoning be effectively treated by the induction of methaemoglobinaemia? Do repeated exposures to H_2S induce sensitization or tolerance to the acute toxicity of the gas?

APPENDIX

TABLE 1. LOSS RIGHTING REFLEX IN EACH GROUP OF MICE

Conc.	Time of Exposure (mins)							
(ppm)	1	2.5	5	7.5	10	12.5	15	30
500								0/20
600						0/20	0/20	5/20
700				0/20	0/20	2/20	3/20	9/20
800	0/20	0/20	0/20	3/20	5/20	9/20	14/20	18/20
900	0/20	0/20	2/20	4/20	6/20	11/20	15/20	16/20
1000	0/20	2/20	6/20	11/20	16/20	16/20	20/20	20/20
1100	1/20	12/20	17/20	20/20	20/20	20/20	19/20	20/20
1200	9/20	17/20	18/20	19/20	20/20	20/20	20/20	20/20
1300	11/20	18/20	18/20	19/20	20/20	20/20	20/20	20/20

TABLE 2. DEATHS IN EACH GROUP OF MICE

Conc.	Time of Exposure (mins)							
(ppm)	1	2.5	5	7.5	10*	12.5	15	30
500								0/20
600						0/20	0/20	0/20
700				0/20	0/20	0/20	0/20	0/20
800		0/20	0/20	0/20	0/46	0/20	0/20	1/20
900		0/20	0/20	0/20	0/46	0/20	2/20	7/20
1000	0/20	0/20	0/20	0/20	9/46	6/20	14/20	12/20
1100	0/20	1/20	4/20	8/20	25/46	13/20	13/20	17/20
1200	0/20	2/20	13/20	14/20	34/46	17/20	19/20	20/20
1300	0/20	3/20	12/20	17/20	44/46	20/20	20/20	20/20

* Values at 10 min exposure represent the combined data of 3 separate experiments.

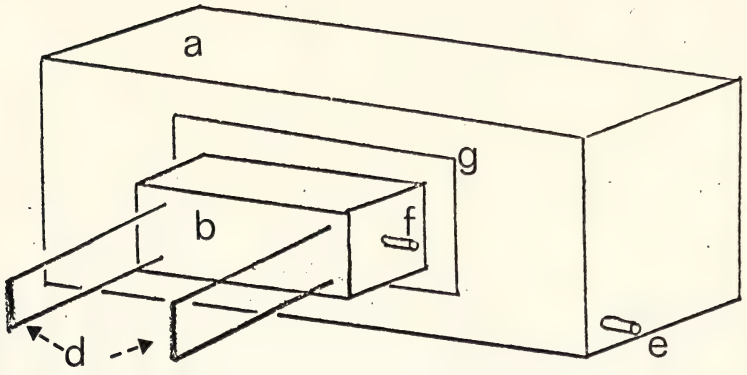
TABLE 3. CONCENTRATION (ppm) OF EXPOSURE (± SD) REQUIRED TO PRODUCE LOSS OF RIGHTING REFLEX AND DEATH OF 50% OF TEST SUBJECTS FOR EACH EXPOSURE DURATION

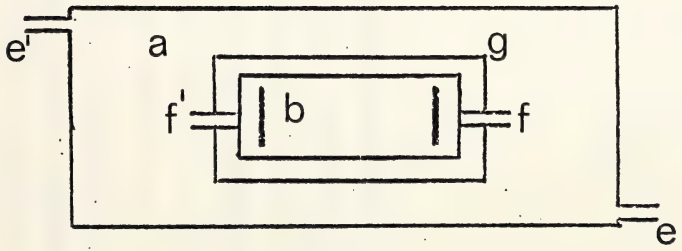
EXPOSURE TIME (mins)	LOSS RR-EC_{50} (SD)	LC_{50} (SD)
2.5	1101 (18)	1734 (110)
5	1039 (19)	1207 (31)
7.5	978 (23)	1132 (25)
10	914 (23)	1097 (13)
12.5	856 (24)	1059 (21)
15	788 (22)	1003 (20)
30	693 (23)	961 (19)

TABLE 4. TIME (MINS) OF EXPOSURE (± SD) REQUIRED TO PRODUCE LOSS OF RIGHTING REFLEX AND DEATH OF 50% OF TEST SUBJECTS FOR EACH EXPOSURE CONCENTRATION

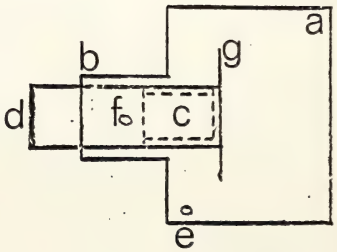
EXPOSURE CONC. (ppm)	ET_{50} (LOSS RR) (SD)	LT_{50} (DEATH) (SD)
700	>30	>30
800	13.2 (1.0)	>30
900	12.7 (1.4)	>30
1000	6.6 (0.7)	18.6 (3.0)
1100	2.4 (0.3)	10.3 (1.2)
1200	1.0 (0.7)	5.2 (1.3)
1300	0.7 (0.9)	4.3 (0.4)

Figure 1. Diagrammatic representation of the gassing apparatus. It consisted of a large (100 x 30 x 40 cm) gassing chamber (a) with a smaller (35 x 12 x 12) entry/exit chamber (b) attached to one side. A wire cage (c) which contained the animals under test could be moved from the entry/exit chamber into the gassing chamber by slides (d). When these slides were in the out position, the wire cage was wholly contained in the entry/exit chamber. In addition, this chamber was automatically sealed from the gassing chamber by a door (g) which was attached to the slides. These slides are shown in an intermediate position in the side view diagram. With the door closed, the entry/exit chamber could be continuously flushed with air (from f to f^1) without affecting the H_2S concentration in the gassing chamber. A continuous flow of H_2S passed through the gassing chamber (from e to e^1). As an addition safeguard, the entire gassing system, including the H_2S supply, was housed within a fume hood installation.





front



side

Figure 2. Graph of % (probability scale) of animals killed (ordinate) at various concentrations (ppm, log scale) of H_2S (abscissa) for different times of exposure (2.5-30 min). Points represent the % killed of each group of 20 animals at each concentration-time combination. Lines between 5 and 95% were drawn by computer regression analysis. Decreasing the time of exposure produced a time dependent shift of the curves to the right, i.e. towards higher concentration levels.

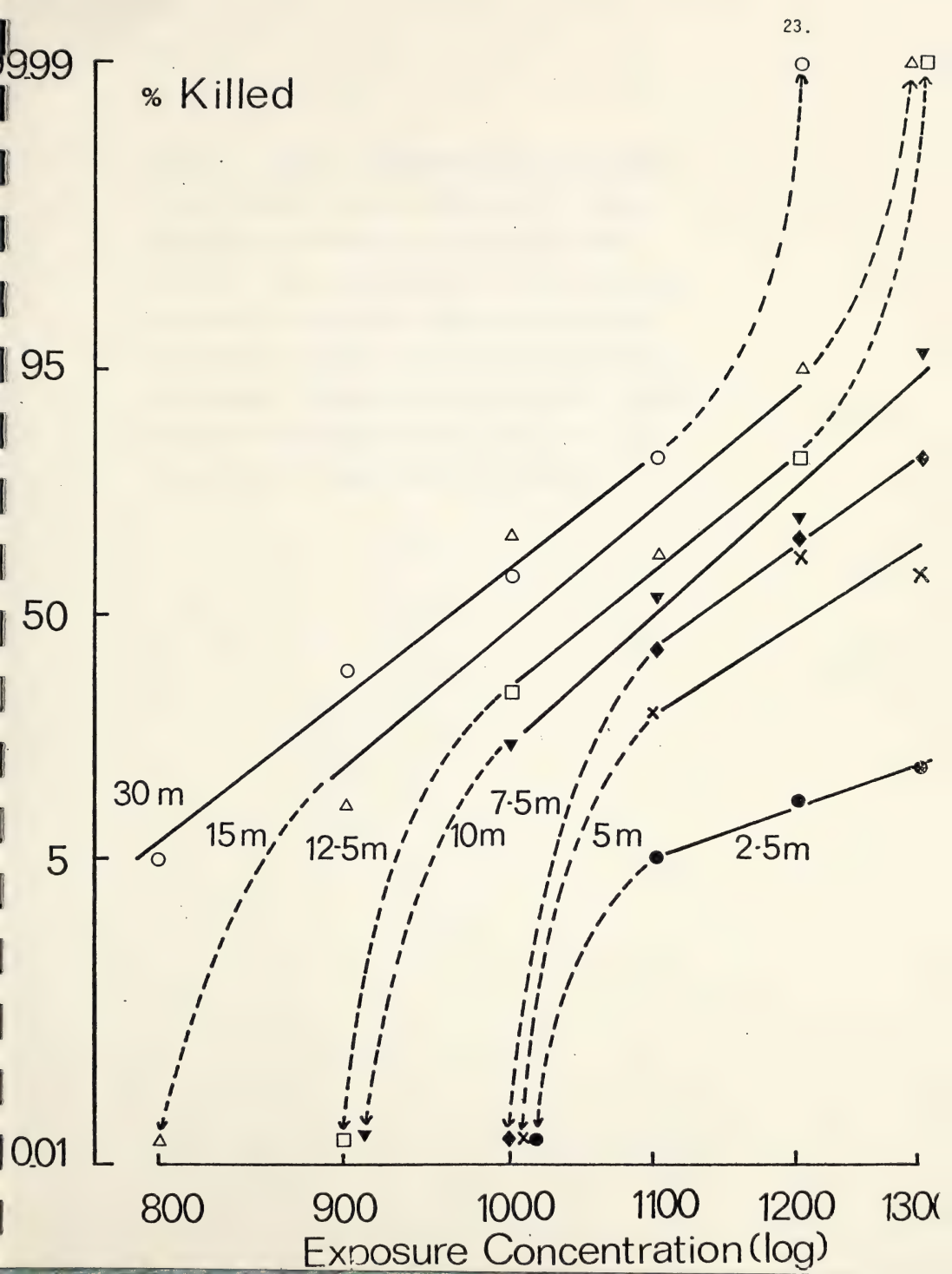

% Killed
99.9
95
50
5
0.01
30 m
15 m
12·5m
10m
7·5m
5m
2·5m
800
900
1000
1100
1200
Exposure Concentration (log)

Figure 3. Graph of % (probability scale) of animals killed (ordinate) at various times (min) of exposure (abscissa) for different H_2S concentrations (800 - 1300 ppm). Points represent the % killed of each group of 20 animals at each time-concentration combination. Lines between 5 and 95% were drawn by computer regression analysis. Increasing the concentration of exposure produced a concentration dependent shift of the curves to the left; i.e. toward shorter time intervals.

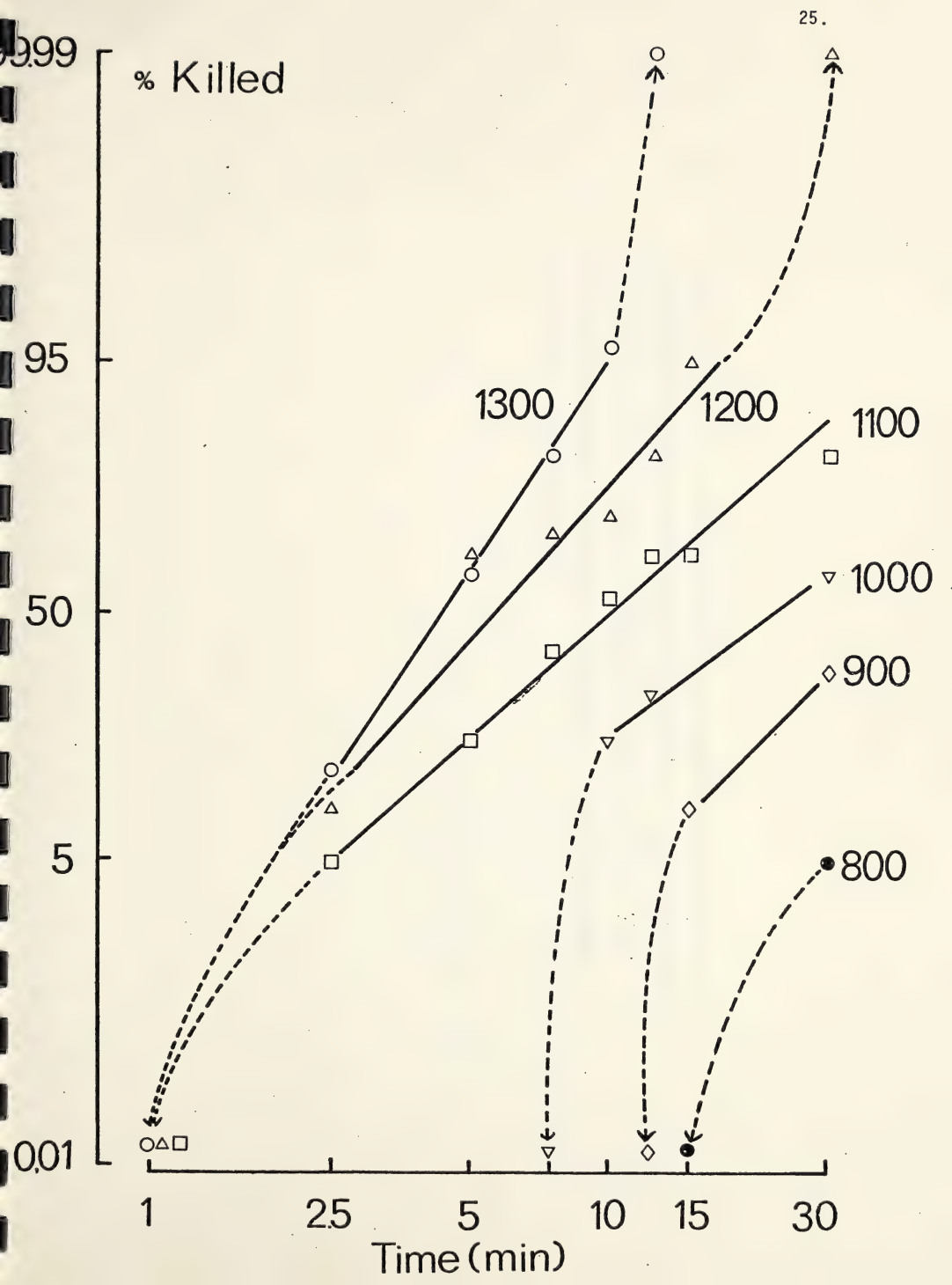

% Killed
99.99
95
50
5
0.01
1300
1200
1100
1000
900
800
1
2.5
5
10
15
30
Time (min)

Figure 4. Graph indicating the effect of the duration (min) of exposure (ordinate) on the LC_{50} concentration of H_2S (abscissa). LC_{50} values were calculated by probit analysis and represent the mean ± SD; $n \geq 60$. (0% and 100% values were not included in the analysis). The LC_{50} was higher at the lower exposure durations.

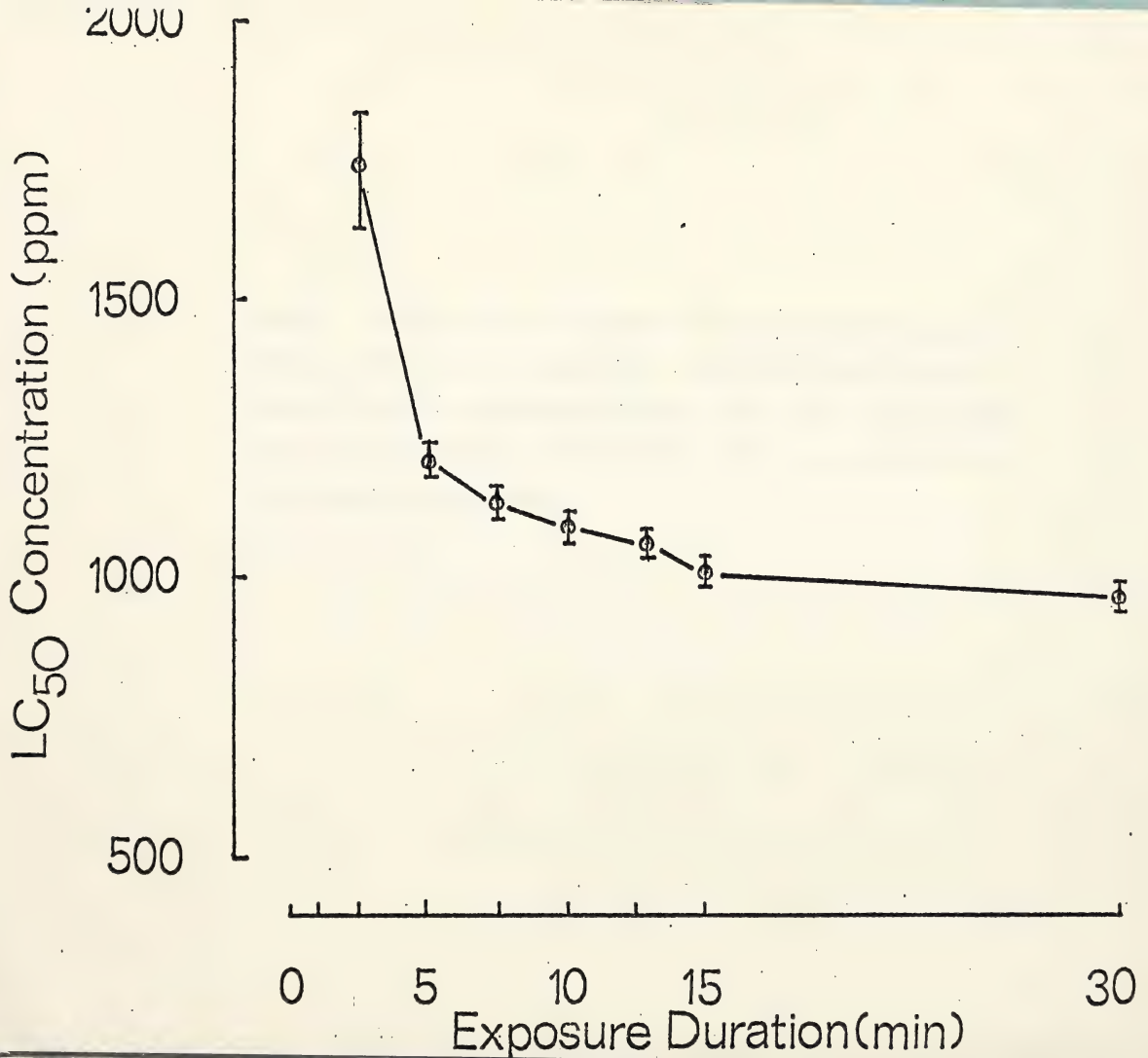
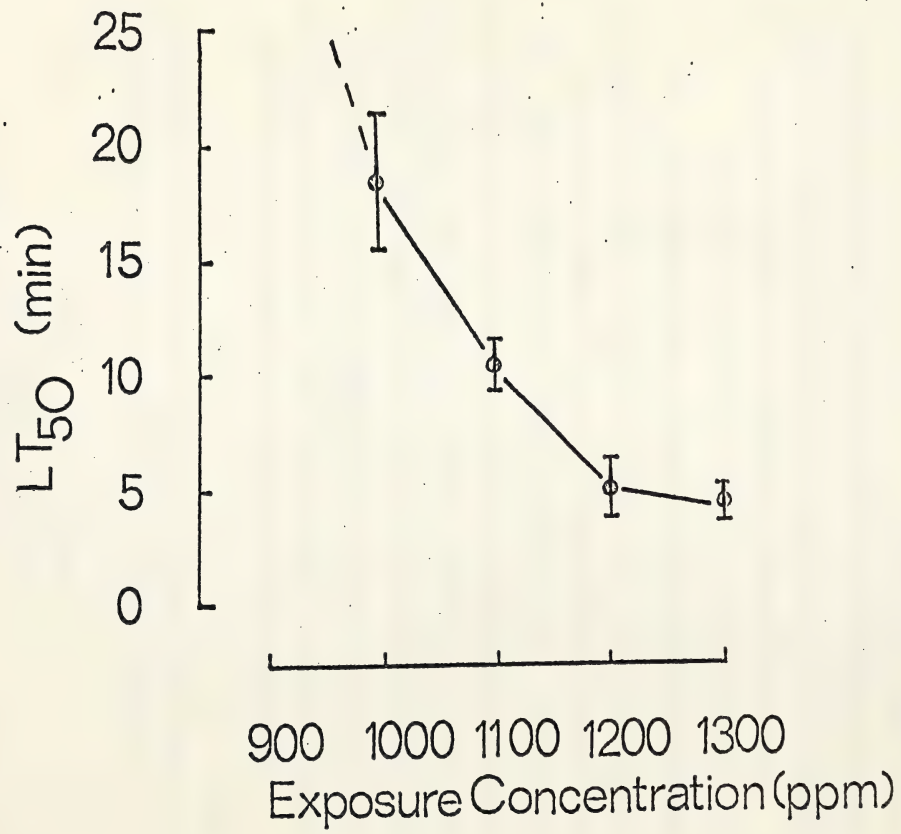

2000
1500
1000
500
LC_{50} Concentration (ppm)
0
5
10
15
30
Exposure Duration (min)

Figure 5. Graph indicating the effect of the concentration (ppm) of exposure (ordinate) on the LT_{50} of H_2S. LT_{50} values were calculated by probit analysis and represent the mean ± SD; $n \geq 60$. (0% and 100% values were not included in the analysis). The LT_{50} was longer at the lower exposure concentrations.


25
20
15
10
5
0
LT_{50} (min)
900 1000 1100 1200 1300
Exposure Concentration (ppm)

AN EXPERIMENTAL INVESTIGATION OF THE LETHALITY OF HYDROGEN SULPHIDE

A. S. Clanachan
Department of Pharmacology
University of Alberta
Edmonton, Alberta T6G 2H7

The potential interaction between the concentration and the duration of exposure on the acute toxicity of hydrogen sulphide (H_2S) was investigated.

Groups of mice (BALB/CCR strain) were exposed to various H_2S concentrations (prepared by dynamic dilution) for seven different time intervals (2.5 - 30 min.). Exposure times and concentrations were randomised. An LC50 value (the concentration of gas which kills 50% of test subjects) was calculated for each exposure duration. The LC50 (± SD; n = 20) at 2.5 min. exposure was 1734 ppm (± 110) whereas following 30 min. exposure the LC50 was 961 (± 19). Death appeared to result from respiratory arrest. Surviving animals recovered rapidly (~ 2 min.), and were retained for a further 5 days. There were no additional deaths. These results indicate that the LC50 is indeed time-dependent - higher concentrations of gas were required to cause death at the shorter exposure durations.

LC50 values, although time-dependent, were confined to a narrow concentration range (961-1734 ppm). However, in the general population, where many factors can influence sensitivity, lethality may extend over greater concentration ranges.

Supported by Alberta Environment.

REFERENCES

Bliss, C.I. (1935). The comparison of dosage mortality data. Ann. Appl. Biol. 22, 307-333.

Cook, D.A. (1972). The use of the APL/360 system in pharmacology II - Probit analysis. Comput. Biomed. Res. 5, 357-350.

Finney, D.J. (1947). Probit Analysis. Cambridge University Press, London.

Haggard, H.W. (1925). Toxicology of hydrogen sulfide. J. Ind. Hyg. Toxicol. 1, 113-121.

O'Donoghue, J.G. (1961). Hydrogen sulfide poisoning in swine. Can. J. Comp. Med. 25, 217-219.

Sayers, R.R., Smith, N.A.C., Gieldner, A.C. et al. (1925). Investigation of toxic gases from Mexican and other high-sulphur petroleums and products. Report by the Bureau of Mines, Department of Interior, to the American Petroleum Institute.

Smith, R.P. and Gosselin, R.E. (1964). The influence of methemoglobinemia on the lethality of some toxic anions; II. Sulfide. Toxicol. Appl. Pharmacol. 6, 584-592.

Smith, R.P. and Gosselin, R.E. (1966). On the mechanism of sulfide inactivation by methemoglobin. Toxicol. Appl. Pharmacol. 8, 1-9-172.

Weber, H.D. and Lendle, L. (1965). Toxische wirkungsbedingungen von Schwefelwasserstoff und Behandlungsmaglichkeiten der akuten Vergiftung. Arch. Toxicol. 20, 290-312.

DATE DUE SLIP

DUE CAM NOV 24 '88
NOV 23 RETURN

DUE CAM NOV 08 '88
OCT 28 RETURN

DUE CAM NOV 04 '89
OCT 22 RETURN

DUE CAM FEB 2 6 '90
MAR 03 RETURN

DUE CAM JUN 12 '90
JUN 08 RETURN

DUE CAM APR 30 1992

DUE CAM APR 30 1993
NOV 06 RETURN

DUE CAM OCT 29 '93

DUE CAM NOV 12 1993
OCT 29 RETURN

DUE CAM APR 29 '94
MAR 04 RETURN

F255